Dose Reconstruction from Urinary Biomarkers

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1 ABSTRACT

The use of biomarkers for human health risk assessment is attractive because they are an indicator of the dose that actually entered the body by all routes. This is an important consideration given the need to include aggregate exposures from diet and other pathways for pesticides. Quantitative relationships between biomarker and environmental concentrations are often unclear, because what is seen in a urine sample depends on the route and time-profile of the exposure. Pharmacokinetic (PK) models describe the dynamics of the chemical in the body. By inverting the appropriate mathematical expressions, the absorbed dose can be calculated from the concentration of the parent compound or a metabolite in a spot urine sample. The goal of this paper is to review the assumptions used in interpreting urinary biomarkers and highlight the role of PK models in reconstructing dose from spot urine measurements. We will demonstrate the estimation method and the impact of different exposure scenarios on the interpretation of the biomarker measurement for chlorpyrifos.

2 INTRODUCTION

- Interpretation of biomarkers essential to estimate aggregate exposure
- Represents the total amount that was absorbed by the body from all routes
- Proper estimation of total absorbed dose enables the evaluation of exposure to dose models
- Requires knowledge about the exposure scenario and the behavior of the chemical in the body

Inhalation
Ingestion
Dermal

Biomarkers

3 Assumptions and Estimates Used for Calculation of Absorbed Dose for Different Exposure Scenarios

	Steady State Mass Balance	Pharmacokinetic (PK) Model for Exposure Events
Assumptions	Constant absorbed dose rate and urinary excretion rate, UER (mass of metabolite excreted into the urine per hour, Fg/hr)	Exposure routes and profiles over time
Model Estimates		PK model parameters estimated from clinical studies: absorption rate constants, **k** [hr-1] elimination rate constant, k_e [hr-1]
		Physiological parameters: body weight, bw [kg] distribution volume, V_d [mL/kg]
		Record/estimate the duration of time since previous void
	Selectivity, S [dimensionless]: fraction of absorbed chemical that is converted to the corresponding urinary metabolite	
	Urinary excretion rate (UER) for the metabolite from spot urine measurement (eliminates variations due to urinary water content)	

4 Two Approaches for Calculating Urinary Excretion Rate (UER [Fg metabolite/hr]) from Spot Urine Measurements (Fg/L or Fg/g creatinine)

Method 1: From urine metabolite concentration (C_u [Fg/L]) and urine volume

a) good when total urine volume (void volume, V_u [L]) and time since last void (t_c–t_s) are recorded; these may be difficult to obtain or unreliable for young children

b) daily/hourly urine output can also be estimated from standard tables

$$\overline{UER}\left[\frac{\mu g}{hr}\right] = \frac{V_u C_u}{t_c - t_s} = \left(\frac{\mu g\ metabolite}{L_{urine}}\right) \times \left(\frac{L_{urine}}{hr}\right)$$

Method 2: From creatinine (cr) corrected metabolite concentration (Fg/g_{cr}) and tabulated (age and body weight specific) standard creatinine production rate

a) more suitable if void volume and time since last urination were not recorded

b) assumes that creatinine production is constant over time; substantial variability was noted in children using method 1 (urine volume)

$$\overline{UER}\left[\frac{\mu g}{hr}\right] = \left(\frac{\mu g\ metabolite}{g_{cr}}\right) \times \left(\frac{g_{cr}}{kg\,bw \bullet hr}\right) \times (bw)$$

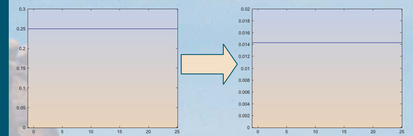
5 Dose Reconstruction from the Calculated UER (Fg/hr): Steady State Assumption

1) Estimate selectivity (S) from clinical experiments

2) Assume a constant absorbed dose rate over time

$$ADr\left[\frac{\mu g}{kg \bullet day}\right] = \left(\frac{\overline{UER}}{bw}\right) \times \left(\frac{mw\ chemical}{mw\ metabolite}\right) \times \left(\frac{1}{S}\right)$$

mw = molecular weight



Calculated UER of metabolite based on urine concentration 0.25 Fg TCPY/hr

Estimated absorbed dose rate of chemical 0.014 Fg chlorpyrifos/kg-hr

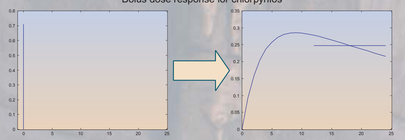
The steady-state model can also be used to approximate a repeating daily dose.

6 Using a PK Model to Estimate Dose from an Exposure Event

Concentration (C_u) → Average Urinary Excretion Rate ($\overline{UER}$) → Absorbed Dose

1) Assume scenario for timing and routes (oral, dermal, inhalation)

Bolus dose response for chlorpyrifos



Absorbed oral dose, 0.71 Fg Chlorpyrifos/kg bw

Calculated UER for metabolite, TCPY, 0.25 Fg/hr

The scenario must be assumed, since several exposure profiles could result in the same calculated UER.

2) The average UER is then incorporated into the PK model by knowing or estimating the duration of time since the previous void

$$\overline{UER} = \frac{1}{t_c - t_s}\int_{t_s}^{t_c}[C_u(\mathbf{t}, \mathbf{k}, \mathbf{V}; dose)]_{model}\,dt = \frac{V_u(C_u)_{measured}}{t_c - t_s}$$

The PK model parameters are the rate constants, **k**, the distribution and compartment volumes, **V**, and the times since exposure and the previous void, **t**

3) After integration of the expression for $(C_u)_{model}$, the dose is then an algebraic function of the measured urine concentration and the void volume

$$dose\left[\frac{\mu g}{kg\,bw}\right] = f[(C_u)_{measured}, V_u, \mathbf{t}, \mathbf{k}, \mathbf{V}]$$

Note that for a linear PK model, the compartment concentrations (**C**) are found from

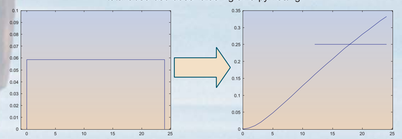
$$\mathbf{C}(t) = \int_{t_0}^{t} e^{-\mathbf{K}(t-s)}\mathbf{I}(s)ds + e^{-\mathbf{K}t}\mathbf{C}_0$$

and the input vector, **I**, and initial conditions, $\mathbf{C_0}$, are functions of dose

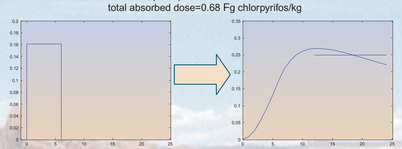
Analytical solutions can be found for simple input profiles (bolus dose, step functions)

Other Possible Inputs to Calculate an Identical UER =0.25 Fg TCPY/hr

square wave input over 24 hrs
total absorbed dose=0.98 Fg chlorpyrifos/kg



square pulse over 6 hours
total absorbed dose=0.68 Fg chlorpyrifos/kg



After 24 hours, the 6-hour square wave response is nearly identical to the bolus dose response.

7 Chlorpyrifos Example: Pharmacokinetic Model (Nolan et al., 1984)

Chlorpyrifos | **TCPY (metabolite)**

intake (Fg/kg-hr) → absorbed dose (Fg/kg) → body conc TCPY (Fg/mL) → urinary excretion rate UER (Fg/hr)

The compartment concentrations are described by $\frac{d}{dt}\begin{pmatrix} C_a \\ C_b \end{pmatrix} = \begin{pmatrix} -k_a & 0 \\ k_a & -k_b \end{pmatrix}\begin{pmatrix} C_a \\ C_b \end{pmatrix} + \begin{pmatrix} I_a \\ 0 \end{pmatrix}$ where I_a is an absorbed dose rate (Fg/kg-day), C_a is the concentration in the intake compartment (skin, lungs, or gi tract), and C_b is the concentration in the blood

Nolan, R.J.; Rick, D.L.; Freshour, N.L.; Saunders, J.H. Chlorpyrifos: Pharmacokinetics in Human Volunteers, *Toxicol. Appl. Pharm.* 1984, 73, 8-15.

8 Estimating Absorbed Dose of Chlorpyrifos

1) Integrate the PK model to solve for the compartment concentrations. The urinary excretion rate is related to the blood concentration by

$$UER = k_e C_b V_d (bw)$$

2) The average UER is obtained by integrating the UER over the time since the previous void. Comparing the model predicted UER to the UER calculated from the biomarker enables the estimation of dose.

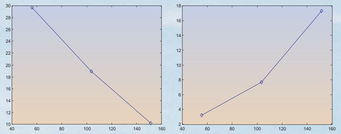
For a bolus that dose occurred at time t_d, the dose can be calculated

$$dose\left[\frac{\mu g}{kg\,bw}\right] = \frac{(k_a - k_e)\left(^{mw_{CPY}}/_{mw_{TCPY}}\right)V_u(C_u)_{measured}}{k_e k_a S(bw)\left[\frac{1}{k_a}e^{-k_a(t-t_d)} - \frac{1}{k_e}e^{-k_e(t-t_d)}\right]_{t_s}^{t_c}}$$

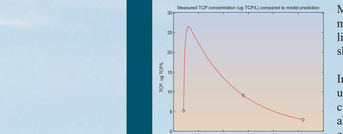
Note that doses from other routes and events are assumed to be additive.

9 MN Children's Pesticide Study Data: Classes of Time Profiles

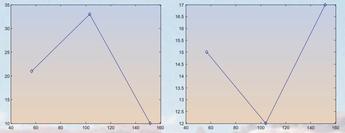
Urine samples (Fg TCPY/L urine) were taken on 3 alternating days (days 3, 5, and 7 of study)



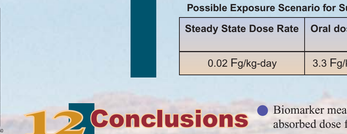
decreasing trend (n=16)



increasing trend (n=15)



maximum on day 5 (n=24)



minimum on day 5 (n=30)

Average within-individual coefficient of variation (SD/$\bar{x}$) is 0.63 (n=85)

Range of COV is 0.04-1.7

Possible steady-state cases (based on a daily repeating dose), COV<0.38, n=24 (28%)

For most cases, steady-state exposure scenario unlikely

10 Comparison of Steady-State Assumption to a Dynamic Scenario Assumption Summary of the MN Children's Study

Averages: bw =30.1 kg TCPY =9.1 Fg/L n=85

	Chlorpyrifos absorbed dose	Mean	Std. Dev.	Percentiles		
				25%	median	75%
SS	Steady-state absorbed dose rate **(Fg/kg-day)**	0.49 (per day)	0.37	0.20	0.37	0.58
PK	Pulse absorbed oral dose occurring 36 hr prior to collecting the morning void (10 hr) **(Fg/kg)**	1.61 (per event)	1.23	0.65	1.22	1.91

Results based on 3 day average of TCPY concentration in urine

Assume 100% conversion of absorbed chlorpyrifos to urinary TCPY

11 Reconstruction of an Oral Dose of Chlorpyrifos

Measured TCPY concentration in urine vs. model prediction. The model is the solid line, and the measured concentrations are shown as ' '.

In this case, the timing of the dose is unknown. The time at which the dose occurred, the magnitude, and the background absorbed dose rate were fit to the model equations by nonlinear optimization.

Possible Exposure Scenario for Subject 442 in the MN Children's Study

Steady State Dose Rate	Oral dose	Time of dose (hours before first sample taken)
0.02 Fg/kg-day	3.3 Fg/kg	8 hours

12 Conclusions

- Biomarker measurements can be used to estimate total absorbed dose from all routes
- Need to know the exposure scenario to interpret biomarker measurements
  - -- Steady-state may not represent actual absorbed dose
  - -- As the duration since exposure increases, the exact profile becomes less important

Future Work

- To determine personal exposure from biomarkers and absorbed dose, additional information is necessary
  - -- Questionnaires
  - -- Diaries
  - -- Environmental measurements